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## Eosinophilic leucocytes and arylsulfatase activity in bronchoalveolar lavage fluid of patients with bronchial asthma.

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## Abstract

The arylsulfatase activity and histamine concentration of bronchoalveolar lavage fluid (BALF) were examined in patients with bronchial asthma in relation to the eosinophil count and asthma type (atopic and non-atopic). The BALF arylsulfatase activity and histamine concentration were significantly higher in atopic asthmatics than in non-atopic asthmatics. In atopic asthmatics, the activity of arylsulfatase was significantly increased in patients with a higher eosinophil count (10% or more). However, the BALF histamine concentration did not correlate with the eosinophil count. In non-atopic asthmatics, there was no significant correlation between arylsulfatase activity and the eosinophil count. The results show that arylsulfatase participates in IgE-mediated allergic reactions.

**KEYWORDS:** eosinophil count, arylsulfatase, histamine, bronchoalveolar lavage fluid, bronchial asthma

## Eosinophilic Leucocytes and Arylsulfatase Activity in Bronchoalveolar Lavage Fluid of Patients with Bronchial Asthma

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The arylsulfatase activity and histamine concentration of bronchoalveolar lavage fluid (BALF) were examined in patients with bronchial asthma in relation to the eosinophil count and asthma type (atopic and non-atopic). The BALF arylsulfatase activity and histamine concentration were significantly higher in atopic asthmatics than in non-atopic asthmatics. In atopic asthmatics, the activity of arylsulfatase was significantly increased in patients with a higher eosinophil count (10% or more). However, the BALF histamine concentration did not correlate with the eosinophil count. In non-atopic asthmatics, there was no significant correlation between arylsulfatase activity and the eosinophil count. The results show that arylsulfatase participates in IgE-mediated allergic reactions.

**Key words :** eosinophil count, arylsulfatase, histamine, bronchoalveolar lavage fluid, bronchial asthma

The mechanism of the onset of bronchial asthma is very complicated. Only two allergic reactions, IgE-mediated (1) and IgG<sub>4</sub>-mediated (2) reactions, have been clearly shown by clinical observation to participate in the onset of bronchial asthma. Although other reactions, for example, IgG-mediated or type III (3) and type IV allergic reactions, have been suggested to participate in the onset of bronchial asthma, their exact role is still unclear.

In IgE-mediated allergic reactions, chemical mediators (mainly histamine, but also leukotrienes) eliciting bronchoconstriction,

are released from mast cells. After tissue reactions are induced by the chemical mediators, white blood cells migrate into the tissue reaction sites and participate in the complex chain of events resulting in the onset of bronchial asthma.

In the present study, the participation of eosinophilic leucocytes in the onset of bronchial asthma was examined in relation to IgE-mediated allergic reactions, by a bronchoalveolar lavage (BAL) method.

### Subjects and Methods

Ten patients with atopic asthma (4 females

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and 6 males), ranging in age from 34 to 67 years (mean, 50.0 years) and 6 patients with non-atopic asthma (so-called intrinsic asthma) (3 females and 3 males), ranging in age from 38 to 58 years (mean, 52.0 years) were selected for this study.

The histamine concentration, arylsulfatase activity and eosinophil count were evaluated in BALF obtained by the previously described bronchoalveolar lavage method (4). The concentration of serum IgE was determined by radio-immunosorbent test (RIST). Histamine was measured by a spectrofluorometric method (5). The albumin concentration was estimated by a Laser-nephelometer PPQ (Hyland). The concentration of histamine in BALF was expressed as the ratio of histamine (ng/ml) to albumin (mg/ml) (Hist/Alb).

Arylsulfatase activity was measured using BALF concentrated 10-fold with Lyphogel. The mixture of 0.5 ml of concentrated BALF, 0.1 ml of 0.3 M sodium acetate buffer (pH 5.0) and 0.4 ml of  $6.25 \times 10^{-3}$  M *p*-nitrocatechol sulfate (*p*NCS) was incubated at 37°C for 1 h. The reaction was stopped by the addition of 67  $\mu$ l of 1N NaOH, and the reaction mixture was centrifuged at 2,000 rpm for 10 min. The *p*-nitrocatechol (*p*NC) concentration of the supernatant was measured with a spectrophotometer (Shimadzu, UV-100-01) at 515 nm (6). The results were expressed as the ratio of arylsulfatase activity ( $\mu$ g *p*NC formed/ml) to albumin (mg/ml) (As/Alb). The eosinophil count in BALF was calculated by differentiating 500 cells, excluding epithelial cells, on a smear preparation. The results were expressed as the percent of eosinophilic leucocytes.

The results were expressed as the mean  $\pm$  SD. The statistical significance of differences between mean values was determined using the unpaired Student's *t* test.

## Results

**Histamine concentration and arylsulfatase activity.** Arylsulfatase activity in BALF of atopic asthmatics (eosinophil count,  $23.8 \pm 22.7\%$ ; serum IgE level,  $1153 \pm 1057$  IU/ml) was  $1.70 \pm 1.54$  As/Alb. The activity in non-atopic asthmatics (eosinophil count,  $10.5 \pm 11.4\%$ ; serum IgE level, 108

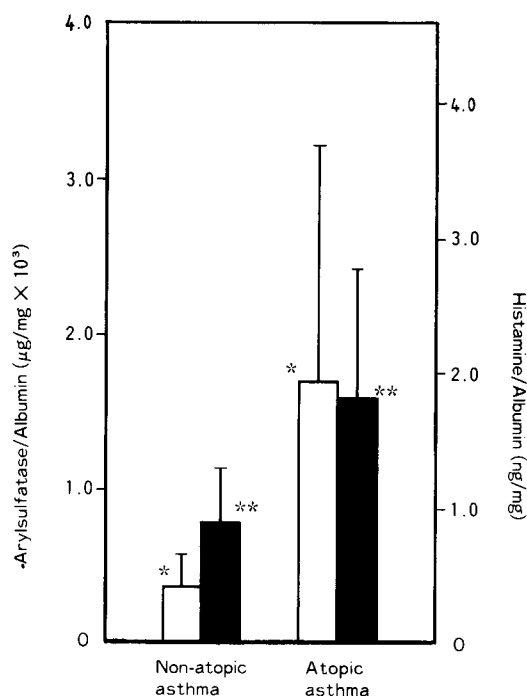


Fig. 1 Arylsulfatase activity (□) and histamine concentration (■) of BALF of non-atopic and atopic asthmatics. The vertical bars represent the standard deviation. \*,  $p < 0.05$ . \*\*,  $p < 0.05$ .

$\pm 60$  IU/ml) was  $0.38 \pm 0.20$  As/Alb. The activity of arylsulfatase in BALF was significantly higher in atopic asthmatics than in non-atopic asthmatics ( $p < 0.05$ ). The histamine concentration of BALF was significantly higher ( $p < 0.05$ ) in atopic asthmatics ( $1.80 \pm 0.94$  Hist/Alb) than in non-atopic asthmatics ( $0.87 \pm 0.42$  Hist/Alb) (Fig. 1).

**Relationship of the eosinophil count to the histamine concentration and arylsulfatase activity of BALF.**

**Atopic asthmatics.** Patients with atopic asthma were divided into two groups according to the BALF eosinophil count (less than 10%, and 10% or more). The BALF histamine concentration of patients with an eosinophil count of 10% or more was  $1.9 \pm 0.6$  Hist/Alb, while the concentration of histamine in patients with an eosinophil count of less than 10% was  $1.73 \pm 1.26$  Hist/Alb. There was no significant difference in

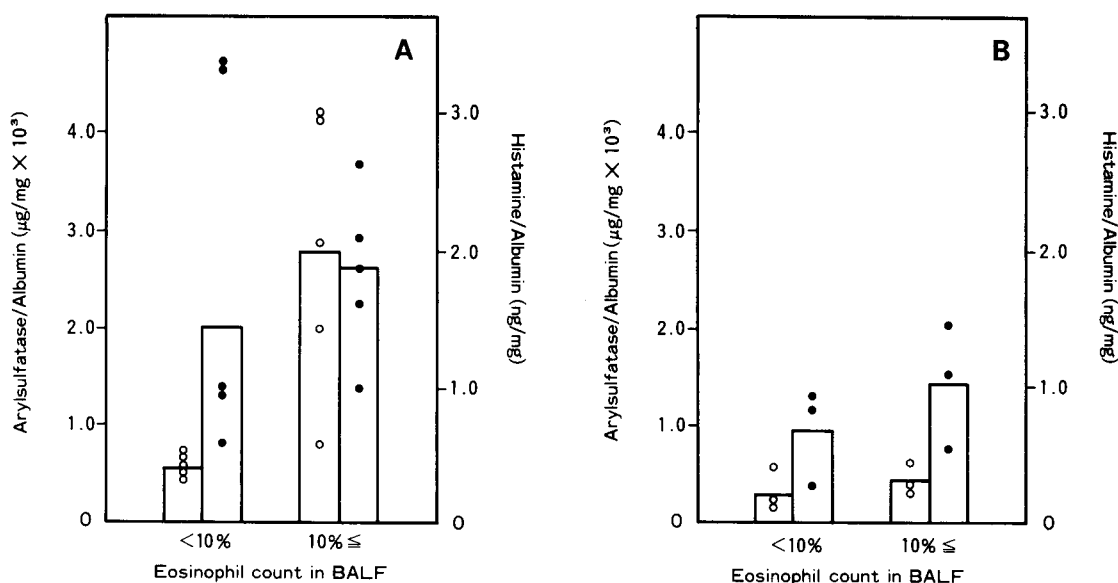


Fig. 2 Arylsulfatase activity (○) and histamine concentration (●) in relation to the eosinophil count of atopic (A) and non-atopic asthmatics (B).

the histamine concentration between the two groups.

Arylsulfatase activity in BALF of patients with an eosinophil count of 10% or more was  $2.8 \pm 1.5$  As/Alb, and the activity in patients with an eosinophil count of less than 10% was  $0.57 \pm 0.11$  As/Alb. The difference in the arylsulfatase activity between the two groups was significant ( $p < 0.01$ ). The results showed that an increase in arylsulfatase activity was accompanied with an increase in the eosinophil count (Fig. 2).

**Non-atopic asthmatics.** Non-atopic asthmatics were divided into two groups in the same manner as atopic asthmatics. The histamine concentration in patients with an eosinophil count of 10% or more was  $1.0 \pm 0.46$  Hist/Alb, and the concentration in patients with an eosinophil count of less than 10% was  $0.69 \pm 0.37$  Hist/Alb. There was no significant difference in the histamine concentration between the two groups. Arylsulfatase activity ( $0.44 \pm 0.17$  As/Alb) in patients with an eosinophil count of 10% or

more was not significantly different from that ( $0.31 \pm 0.24$  As/Alb) in patients with an eosinophil count of less than 10% (Fig. 2).

## Discussion

The IgE-mediated allergic reaction has been clearly shown to participate in the onset of bronchial asthma (1). IgE-mediated allergic reactions comprise two inflammatory responses: the release of chemical mediators (humoral phase) followed by cellular migration (cellular phase) (7).

An attack of atopic asthma is thought to be initiated by the interaction of antigen-IgE antibodies on the surface of tissue mast cells, followed by the release of bronchospasm-eliciting (histamine, leukotrienes, platelet activating factor, *etc*) and cell-attracting (eosinophil chemotactic factor, neutrophil chemotactic factor, *etc*) chemical mediators. After the release of chemical mediators, cells migrate into the allergic reaction sites.

Histamine is released from tissue mast cells and blood basophils when the cells are stimulated by antigen and anti-IgE. Our previous studies have shown that the release of histamine from basophils of atopic asthmatics is much higher than that of non-atopic asthmatics (8). The participation of histamine in atopic asthma was also indicated by the present study in that the histamine concentration of BALF of atopic asthmatics was significantly higher than that of non-atopic asthmatics. Furthermore, arylsulfatase activity in BALF was significantly higher in atopic asthmatics than in non-atopic asthmatics. These results revealed that the release of histamine and arylsulfatase closely correlate with IgE-mediated allergic reactions.

Eosinophils are one of the main cells participating in IgE-mediated allergic reactions and the release of arylsulfatase. The results obtained here showed that eosinophils release a fairly large amount of arylsulfatase in IgE-mediated allergic reactions, but not in non-IgE-mediated reactions.

For a long time, arylsulfatase has been considered to inactivate the slow-reacting substance of anaphylaxis (SRS-A), one of the main bronchoconstrictors in bronchial asthma. However, it was shown that SRS-A activity is derived from the action of sulfid-peptide leukotrienes (leukotriene C<sub>4</sub>, D<sub>4</sub> and E<sub>4</sub>) (9), and subsequently it was found that arylsulfatase does not inactivate sulfid-peptide leukotrienes. Therefore, at the present time, the action of arylsulfatase in IgE-mediated allergic reactions is still unclear.

However, the fact that arylsulfatase activity is increased in BALF of atopic asthmatics suggests that the substance plays an important role in IgE-mediated allergic reactions.

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